Association of color and feeding deterrence by tropical reef fishes

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Summary. While many marine molluscs have been suggested to use aposematic coloration to avoid predation, few studies have tested the ability of marine predators to learn to associate colors with distasteful prey. In field experiments, we tested the ability of two populations of reef fishes to discriminate among red, yellow, and black artificial nudibranch models when one color was paired with a feeding deterrent. We offered fishes (1) the models without any feeding deterrents, (2) the models with a feeding deterrent coated onto one color, and (3) the models without deterrents again. If reef fishes learn to associate colors with noxious prey, we expected the color paired with the feeding deterrent to be eaten less frequently in the final assay than the initial assay. In both populations, fishes formed clear associations between color and feeding deterrence. However, when the experiment was repeated in one population, changing the color paired with the feeding deterrent, fishes did not form an association between color and feeding deterrence. In this case, prior learning may have affected subsequent trials. Our study indicates that common colors of nudibranchs are recognizable by fishes and can be associated with noxious prey.

Key words. aposematic coloration – chemical defense – fish feeding behavior – learned aversion – nudibranch

Introduction

Aposematic coloration has been defined as the exhibition of conspicuous color patterns by prey to advertise noxious properties to potential predators (Guilford 1990). This definition implies that conspicuous colorations increase the ability of predators to learn to associate color patterns with distasteful prey. Several authors have shown that conspicuousness does indeed enhance the effectiveness of learning. Gittleman & Harvey (1980) demonstrated that chicks learn aversions to conspicuous prey more readily than to cryptic prey. Gaudy coloration directly affects the strength of initial learning by chicks and the duration of memory for a noxious stimulus (Roper & Redston 1987).

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Research on aposematism in terrestrial animal behavior has been focused mainly on bird–insect interactions (Brower 1984; Cardoso 1997). In marine environments, it is generally accepted that many marine molluscs with conspicuous colorations use distasteful chemicals to deter predatory fishes. The loss of the shell in the evolution of the opisthobranchs has been accompanied by considerable radiations of body shape and color (Faulkner & Ghiselin 1983; Rudman 1991). Edmunds (1991) found strong evidence for aposematism in nudibranch species that are (1) conspicuously colored in their natural habitat, (2) unpalatable to potential predators, and (3) are part of a mimetic group of species. In addition, Edmunds (1991) specified four criteria necessary to demonstrate that a particular species is aposematic: (1) it is distasteful, such that some potential predators do not eat it; (2) it is conspicuously colored; (3) some predators avoid attacking it because of this coloration; and (4) conspicuous coloration provides better protection than would crypsis or other signals.

While many authors have generally accepted the existence of aposematic coloration in marine molluscs, these features have been described from a human perspective (Ros 1977; Thompson 1985; Brunckhorst 1991; Rudman 1991). Color patterns that are conspicuous to the human eye may be quite inconspicuous to the most dangerous predators, which may view the pattern under very different conditions (Endler 1978). In water, different wavelengths of visible light penetrate to different depths. Red light is quickly absorbed, while blue light penetrates deepest (McFarland 1991). To conduct research on the visibility of an organism's color pattern, we must consider not only the inherent properties of the pattern, but also the predator's vision, hunting tactics, prey behavior, and background color patterns. The effects of these factors are not independent, and may vary with depth and location (Endler 1978). Coral reef fishes possess at least two or more visual pigments in their cone cells (Loew & Lythgoe 1985), but the existence of multiple cone visual pigments does not in itself prove that they function to distinguish color; rather, this ability must be documented by quantitative behavioral experiments (McFarland 1991).

Few papers focus on the determinants of color perception for marine animals. Tullrot & Sundberg (1991) report a feeding experiment with cod as a poten-

Table 1 First experimental sequence. Each assay is coded by the series, experimental day, consecutive assay number in the series and treatment, and sample size

Series	Day	Assay	Model	n
1	1	1	BYR	19
1	1	2	BY*R	17
1	1	3	BY*R	7
1	1	4	BYR	10
2	6	1	BYR	20
2	6	2	BYR*	20
2	6	3	BYR*	9
2	6	4	BYR	19
3	13	1	BYR	20

* Color model coated with *Cacospongia* extract

Table 2 Second experimental sequence. Each assay is coded by the series, experimental day, consecutive assay number in the series and treatment, and sample size

Series	Day	Assay	Model	n
4	1	1	BYR	20
4	1	2	BYR*	20
4	1	4	BYR	20
5	6	1	BYR	20
5	6	2	BY*R	20
5	6	3	BY*R	10
5	6	4	BYR	20
6	13	1	BYR	20

* Color model coated with *Cacospongia* extract

tial predator of the nudibranch *Polycera quadrilineata*. The nudibranch was distasteful, was conspicuous in its environment, and was attacked by a predator capable of associating color with distastefulness and remembering this association. Since these results meet the conditions of Edmunds (1991), Tullrot & Sundberg (1991) concluded that this mollusc is aposematic. Likewise, Marin *et al*. (1994) concluded that the color pattern of *Hypselodoris* is aposematic by conducting similar experiments with artificial prey models and the wrasse *Thalassoma pavo* as a potential predator. The wrasse was able to recognize not only the color pattern but also the design of the model (light lines in a dark background).

In order to demonstrate the ability of reef fishes to discriminate colors and to associate colors with noxious prey, we tested the acceptance of colored (black, yellow, and red) artificial nudibranch models by two different reef fish communities. We designed a field experiment involving the three color models in a sequence of treatments in which we offered fishes (1) the three models, (2) the three models with a known feeding deterrent coated onto one color of model, and (3) the three colors of models without deterrents again. If reef fishes can learn to associate colors with noxious prey, we expected the color paired with the feeding deterrent to be eaten less frequently in the final assay than in the initial assay.

Table 3 Third experimental sequence. Each assay is coded by the series, experimental day, consecutive assay number in the series and treatment, and sample size

Series	Day	Assay	Model	n
7	1	1	BYR	20
7	1	2	BYR*	20
7	1	4	BYR	20
8	13	1	BYR	20

* Color model coated with *Cacospongia* extract.

Materials and methods

Artificial nudibranch models

Three colors of nudibranch models were designed for these experiments: red, yellow, and black. Each model consisted of two parts, the main body and the outer skin. The outer skin consisted of two pieces of printed color paper 1.5×3 cm (Sanchez-Jerez *et al*. 1994). The main body was an artificial diet made from 10 g of ground catfish food, 5 g of carrageenan (Sigma 1013), 2 g of agar, and 160 ml of water. The ingredients were mixed by stirring, then heated in a microwave oven for 75 s. The papers were placed onto the diet while it was still warm. We used *Cacospongia* extracts to coat the distasteful models, since scalaradial and desacetyl scalaradial (the main secondary metabolites of *Cacospongia*) are known feeding deterrents to reef fishes (Avila & Paul 1997) and are found in nudibranchs that feed on *Cacospongia*. We used the sponge extract because it was readily available in large quantities, while nudibranch extract was not. We used 620.5 mg of extract for each treatment, or 517 µg/model, dissolved in diethyl ether and painted onto the sides of the models using a microsyringe.

Experimental design

We conducted three experimental sequences, two at the same site in a reef lagoon, but 3 months apart, and a third at a different reef.

First experimental sequence (Table 1)
This experiment was conducted on a small patch reef at 2 m depth in a shallow lagoon, Piti Bombhole, Guam (13.28.23N, 144.42.10E). The fishes observed feeding during the assays were *Abudefduf sexfasciatus*, *Siganus argenteus*, *Amblyglyphidodon curacao*, *Chaetodon auriga*, *C. unimaculatus*, and *C. ulietensis*. All of these fishes are very common in reef lagoons and sheltered bays on Guam (Jones & Chase 1975; Anderston *et al*. 1981; Myers 1991; Meyer & Paul 1995).

To measure avoidance learning in fish communities, we designed a feeding preference experiment using successive feeding assays. We offered fishes a set of three ropes, with different color models on each rope; red, yellow, black. Each rope had four models of the same color attached by safety pins and different ropes had different color models. The three ropes were anchored to the reef within 0.5 m of each other, and each set of three ropes was replicated ($n = 7$ to 19). Each set of ropes was observed until approximately 6 of the 12 models had been eaten. The number of models consumed was recorded for each color.

On Day 1 of the first experimental sequence, we first conducted an initial color preference assay. All of the color models were offered without any feeding deterrents (Trial 1-1-1, $n = 19$). 45 min later, we conducted a stimulus presentation assay. We repeated the first assay, but the yellow color models were coated with *Cacospongia* extract (Trial 1-1-2, $n = 17$). 45 min later, we repeated the stimulus presentation assay (Trial 1-1-3, $n = 7$). Finally, we repeated the initial color preference assay without any feeding deterrents on the models (Trial 1-1-4, $n = 10$).

On Day 6, we repeated the experimental sequence, but coated the red color models with *Cacospongia* extract instead of the yellow models. First, we conducted an initial color preference assay. All of the color models were offered without any feeding deterrents (Trial 2-6-1, $n = 20$). 45 min later, we conducted a stimulus presentation assay. We repeated the first assay, but the red color models were

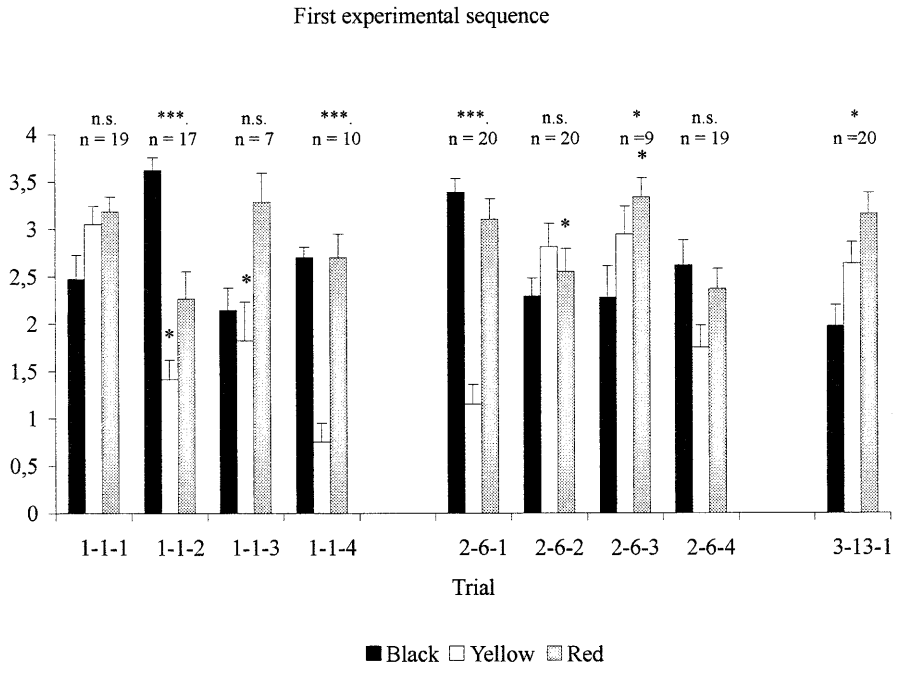

Fig. 1 The number of black, yellow, and red models consumed by reef fishes in the first experimental sequence. Trial code (x-y-z) represents: x: series number; y: experimental day, z: assay number during each series. *: color model coated with *Cacospongia* extract. In series 1, we offered fishes the three color models (assay 1), *Cacospongia* extract coated onto yellow models (assays 2 and 3), and the models without deterrents (assay 4). In series 2, we offered fishes the three color models (assay 1), *Cacospongia* extract coated onto red models (assay 2 and 3), and the models without deterrents (assay 4). In series 3, we offered fishes the three color models without deterrents. Data are means $\pm$ SE, numbers above the bars denote sample size. Statistical significance was determined using Hotelling's T2 test: *: $p < 0.05$; **: $p < 0.01$; ***: $p < 0.001$

coated with *Cacospongia* extract (Trial 2-6-2, n = 20). 45 min later, we repeated the stimulus presentation assay (Trial 2-6-3, n = 9). Finally, we repeated the initial color preference assay without any feeding deterrents on the models (Trial 2-6-4, n = 19).

On Day 13, we conducted another assay to determine if the previous avoidance learning had been retained, offering all three color models without any feeding deterrents (Trial 3-19-1, n = 20).

Second experimental sequence (Table 2)

We repeated the previous experimental sequence 99 days later at the same location. For this sequence, we added *Cacospongia* extract to the red models before the yellow models.

On Day 1, we conducted an initial color preference assay. All of the color models were offered without any feeding deterrents (Trial 4-1-1, n = 20), 45 min later, we conducted a stimulus presentation assay. We repeated the first assay, but the red color models were coated with *Cacospongia* extract (Trial 4-1-2, n = 20). 45 min later, we repeated the stimulus presentation assay (Trial 4-1-3, n = 10). Finally, we repeated the initial color preference assay without any feeding deterrents on the models (Trial 4-1-4, n = 20).

On Day 6, we repeated the experimental sequence, but coated the yellow color models wtih *Cacospongia* extract instead of the red models. First, we conducted an initial color preference assay. All of the color models were offered without any feeding deterrents (Trial 5-6-1, n = 20). 45 min later, we repeated the stimulus presentation assay. We repeated the first assay, but the yellow color models were coated with *Cacospongia* extract (Trial 5-6-2, n = 20). 45 min later, we repeated the stimulus presentation assay (Trial 5-6-3, n = 10). Finally, we repeated the initial color preference assay without any feeding deterrents on the models (Trial 5-6-4, n = 20).

On Day 13, we conducted an assay to determine if the previous avoidance learning had been retained, offering all three color models without any feeding deterrents (Trial 6-13-1, n = 20).

Third experimental sequence (Table 3)

We conducted a third experimental sequence at Western Shoals, Apra Harbor, Guam (13.27.1N, 144.39.6E), a 2 m deep reef formed by a complex mixture of coral patches and rocky boulders near a deep cliff. The dominant fishes observed feeding at this location were *Pomacentrus amboinensis*, *Abudefduf sexfasciatus*, *Ambyglyphidodon curacao*, *Naso vlamingii*, *Cheilinus fasciatus*, *Scarus schlegeli*, and *Thalassoma lutescens*. This population had a higher diversity and was composed of fishes typical of deeper lagoons and seaward reefs (Myers 1991). Time between assays was shortened to 30 min.

On Day 1, we conducted an initial color preference assay. All of the color models were offered without any feeding deterrents (Trial 7-1-1, n = 20). 30 min later, we conducted a stimulus presentation assay. We repeated the first assay, but the red color models were coated with *Cacospongia* extract (Trial 7-1-1, n = 20). 30 min later, instead of repeating the stimulus presentation assay, we offered 30 red models coated with *Cacospongia* extract to surrounding fishes by distributing the models in the water column without anchoring them to ropes (Trial 7-1-3). Finally, we repeated the initial color preference assay without any feeding deterrents on the models (Trial 7-1-4, n = 20).

On Day 13, we conducted another assay to determine if the previous avoidance learning had been retained, offering all three color models without any feeding deterrents (Trial 8-13-1, n = 20).

Statistical analysis

The feeding preference experiments described above are multi-choice assays performed on the same fish community. Thus, with this design we face a two-fold problem of non-independence of variables when analysing the data: consumption of one type is not independent of consumption of other types held together (Peterson & Renaud 1989) and preferences of the fish community at the end of the experiment are not independent of preferences at the beginning (Cardoso 1997). This kind of design can not be analyzed by usual procedures (parametric and non-parametric analysis of variance, t-test) (Peterson & Renaud 1989; Roa 1992).

While several authors have chosen to present their data without statistical analysis (Hay *et al.* 1986), in our case we follow Roa (1992) and use a multivariate analysis technique as follows. If p food types are offered to a fish community in the field (in our case, p = 3, and n = 7 to 20 replicates), then the response variable in such an experiment is a vector X of mean consumptions of dimension p, in which the components (the differently colored models) are correlated. The null hypothesis to be tested is that there is no food preference of the consumer, which is equivalent to testing whether the components of the mean vector X are all equal to a constant k (the overall mean). The test statistic to test this hypothesis is the one-sample Hotelling's $T2 = n(X - k)'S - 1(X - k)$ (where $S - 1$ is the inverse of the sam-

Trial code	N	Centered mean			T^2	df	F	p
		black	yellow	red				
1,1,1	19	−0.430	0.149	0.280	0.283	3,16	1.509	0.250
1,1,2	17	1.187	−1.019	−0.166	7.517	3,14	35.080	0.000
1,1,3	7	−0.274	−0.596	−0.869	2.201	3,4	2.934	0.163
1,1,4	10	0.650	−1.300	0.650	10.421	3,7	24.316	0.000
2,6,1	20	0.842	−1.396	0.554	4.372	3,17	24.772	0.000
2,6,2	20	−0.263	0.263	−0.000	0.196	3,17	1.109	0.373
2,6,3	9	−0.574	0.093	0.481	2.570	3,6	5.140	0.043
2,6,4	19	0.372	−0.496	0.122	0.386	3,16	2.061	0.146
3,13,1	20	−0.617	0.045	0.571	0.756	3,17	4.286	0.020

Table 4 Result of Hotelling's T2 test on data from feeding preference of reef fishes in the first experimental sequence. Trial code (x, y, z) represents: x: series number; y: experimental day, z: assay number during each series. Centered mean (X − k) is the vector of differences between the sample means and the hypothesized constant k (the overall mean)

pling variance–covariance matrix, and (X − k) is the vector of differences between the sample means and the hypothesized constant). pling variance–covariance matrix, and (X − k) is the vector of differences between the sample means and the hypothesized constant). When the null hypothesis is true, F = [(n − p)/(p(n − 1))] and T2 has the F distribution with p and n − p degrees of freedom. When the null hypothesis of no preference is rejected, those food types with means greater than the constant k are suspected to be preferred, and the opposite for foods with means less than k. In order to simplify the identification of preferred and rejected food types, we centered the data around zero, subtracting the constant k from each datum, so that the null hypothesis of no preference is whether the components of the mean vector are equal to zero. We performed distinct analyses for each assay, due to the time-dependence of the data. Since no autogenic changes in the absence of consumers are expected to occur in the models during the experiments, we do not include controls (Roa 1992). All the calculations were done using SYSTAT v. 5 (Wilkinson 1988).

Results

The *Cacospongia* extract was an effective feeding deterrent in the trials with extract coated onto yellow models (Trial 1-1-2: $T^2 = 7.517$, $F_{3,14} = 35.08$, $p = 0.000$; Trial 5-6-2: $T^2 = 1.612$, $F_{3,17} = 9.136$, $p = 0.001$; Trial 5-6-3: $T^2 = 1.949$, $F_{3,7} = 4.548$, $p = 0.045$) and red models at Western Shoals (Trial 7-1-2: $T^2 = 0.783$, $F_{3,17} = 35.08$, $p = 0.018$). However, the extract was not deterrent when coated onto red models at Piti Bombhole (Trial 2-6-2: $T^2 = 0.196$, $F_{3,17} = 1.11$, $p = 0.373$; Trial 4-1-2: $T^2 = 0.03$, $F_{3,17} = 0.189$, $p = 0.902$).

In the first trial (Fig. 1, Table 4) at Piti Bombhole (Trial 1-1-1: $T^2 = 0.283$, $F_{3,16}$: 1.509, $p = 0.250$), there were no clear preferences among the three colors. After we offered yellow models with *Cacospongia* extract, fishes consumed significantly fewer yellow models and more black models (Trial 1-1-2: $T^2 = 7.517$, $F_{3,14} = 35.08$, $p = 0.000$). The negative centered mean value for yellow models indicates that significantly fewer yellow models were consumed, while the positive mean value for black models indicates that significantly more black models were consumed (Table 4). The same preferences were observed when the models were not coated with extract (Trial 1-1-4: $T^2 = 10.421$, $F_{3,7} = 24.316$, $p = 0.000$) and these preferences remained a week later (Trial 2-6-1: $T^2 = 4.372$, $F_{3,17} = 24.772$, $p = 0.000$). After offering red models with *Cacospongia* extract, consumption of the yellow models increased significantly, but consumption of the red and black models did not change (Trial 2-6-2: $T^2 = 0.196$, $F_{3,14} = 1.109$, $p = 0.373$). After an additional week, consumption of yellow models increased, consumption of black models decreased, and consumption of red models did not change (Trial 3-13-1: $T^2 = 0.756$, $F_{3,17} = 4.286$, $p = 0.020$). Fishes consumed less of the yellow models after they were associated with feeding deterrents, and retained this association for 5 days. However, fishes did not change their consumption of red models when they were associated with feeding deterrents (Fig. 1, Table 4).

In the second experimental sequence, (Fig. 2, Table 5), initial relative consumption was similar to the first experimental sequence with approximately equal amounts of each color model eaten (Trial 4-1-1: $T^2 = 0.125$, $F_{3,17} = 0.709$, $p = 0.560$). After presenting the red models with feeding deterrents, fishes showed no change in relative consumption of the three models (Trial 4-1-2: $T^2 = 0.033$, $F_{3,17} = 0.189$, $p = 0.902$). However, a week later there was a significant decrease in consumption of the black model (Trial 5-6-1: $T^2 = 0.685$, $F_{3,17} = 3.881$, $p = 0.028$). After we offered yellow models coated with *Cacospongia* extracts, consumption of the yellow models did not change, while consumption of the black models significantly decreased and consumption of the red models significantly increased (Trial 5-6-4: $T^2 = 7.75$, $F_{3,17} = 43.938$, $p = 0.000$). One week later, consumption of black models increased significantly and consumption of red models decreased significantly, with no change in consumption of the yellow models (Trial 6-13-1: $T^2 = 2.706$, $F_{3,17} = 15.334$, $p = 0.000$). The final relative preferences were different from the initial trial, with significantly greater consumption of black models and less consumption of yellow models (Fig. 2, Table 5). Thus, in the second experimental sequence, we found no association of color with feeding deterrence.

In the third experimental sequence at Western Shoals, the initial relative preferences were different from the Piti Bombhole site (Fig. 3, Table 6), with greater consumption of red color models (Trial 7-1-1: $T^2 = 1.83$, $F_{3,17} = 10.371$, $p = 0.000$). When we offered the red toxic models, we observed a significant increase in consumption of the black models and a decrease in consumption of the red models (Trial 7-1-2: $T^2 = 0.783$ $F_{3,17} = 4.437$, $p = 0.018$). One week later, the relative preferences returned to their initial state, with a significant increase in consumption of red models (Trial 8-6-1:

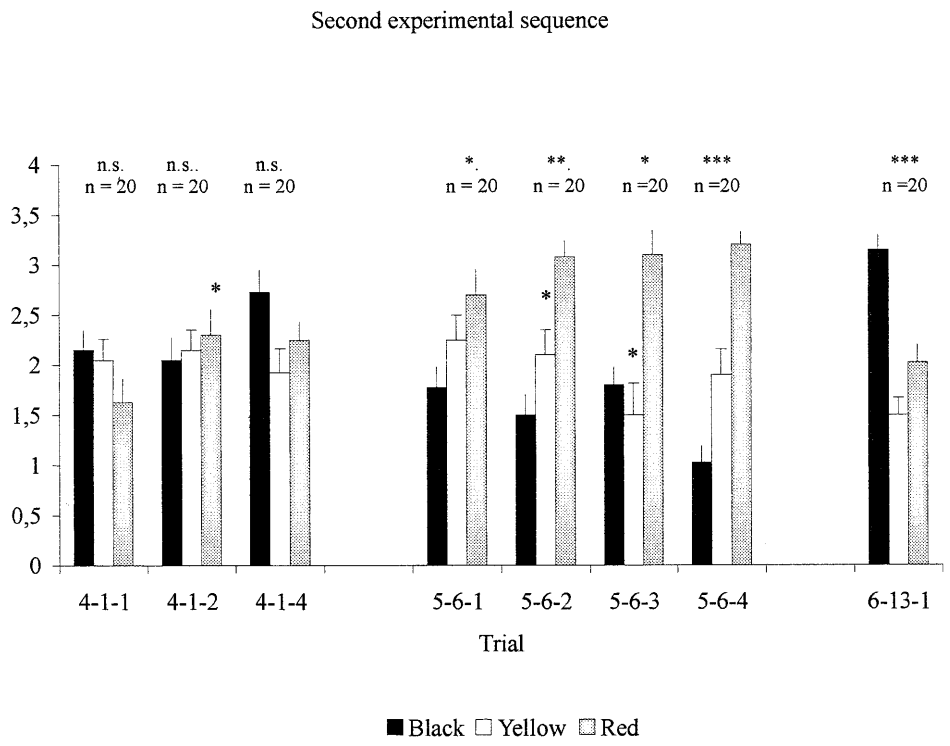

Fig. 2 The number of black, yellow, and red models consumed by reef fishes in the second experimental sequence. Trial code (x-y-z) represents: x: series number; y: experimental day, z: assay number during each series. *: color model coated with *Cacospongia* extract. In series 4, we offered fishes the three color models (assay 1), *Cacospongia* extract coated onto red models (assay 2), and the models without deterrents (assay 4). In series 5, we offered fishes the three color models (assay 1), *Cacospongia* extract coated onto yellow models (assay 2, 3), and the models without deterrents (assay 4). In series 6, we offered fishes the three color models without deterrents. Data are means $\pm$ SE, numbers above the bars denote sample size. Statistical significance was determined using Hotelling's T2 test: *: $p < 0.05$; **: $p < 0.01$; ***: $p < 0.001$

$T^2 = 0.9$, $F_{3,17} = 5.098$, $p = 0.011$, Fig. 3, Table 6). This experiment showed a clear association between red color models and feeding deterrence, but fishes did not retain this association after 12 days.

Discussion

Nudibranchs can sequester some of the secondary metabolites produced by their dietary sponges (Rogers & Paul 1991). Scalaradial has been considered a distasteful chemical that acts as a predator deterrent in sponges and nudibranchs (Cimino *et al.* 1982; Rogers & Paul 1991; Avila & Paul 1997). The tasting and subsequent rejection of the extract-coated models by reef fishes in our experiments confirm that the compounds found in *Cacospongia* (mostly scalaradial, desacetyl scalaradial, and scalarin) can be strong feeding deterrents. The diversity of fishes involved in the experiments suggests that animals containing these metabolites are avoided by most of their potential predators.

We found differences in the initial relative color preferences between two assemblages of reef fishes. Decisions about whether or not to eat a particular food may be affected by previous experiences with any poisons or other harmful substances that may be present in such food (Broom 1981). The Western Shoals population showed a strong initial preference for red models, while no strong preferences were found at Piti Bombhole. This difference could be due to experiences with potential prey having these colors, which could differ between the two locations. The widespread association between noxious qualities and warning coloration in animals appears to have arisen because striking colors are more easily remembered by predators than cryptic ones (Gittleman & Harvey 1980; Gittleman *et al.* 1980).

Trial code	*N*	Centered mean			T^2	*df*	*F*	*p*
		black	yellow	red				
4,1,1	20	0.208	0.208	−0.317	0.125	3,17	0.709	0.560
4,1,2	20	−0.117	−0.117	0.133	0.033	3,17	0.189	0.902
4,1,4	20	0.425	−0.375	−0.050	0.287	3,17	1.624	0.221
5,6,1	20	−0.642	−0.167	0.283	0.685	3,17	3.881	0.028
5,6,2	20	−0.725	−0.125	0.850	1.612	3,17	9.136	0.001
5,6,3	10	−0.333	−0.633	0.976	1.949	3,7	4.548	0.045
5,6,4	20	−1.017	−0.142	1.158	7.75	3,17	43.938	0.000
6,13,1	20	0.925	−0.725	−0.200	2.706	3,17	15.334	0.000

Table 5 Result of Hotelling's T2 test on data from feeding preference of reef fishes in the second experimental sequence. Trial code (x, y, z) represents: x: series number; y: experimental day, z: assay number during each series. Centered mean (X − k) is the vector of differences between the sample means and the hypothesized constant k (the overall mean)

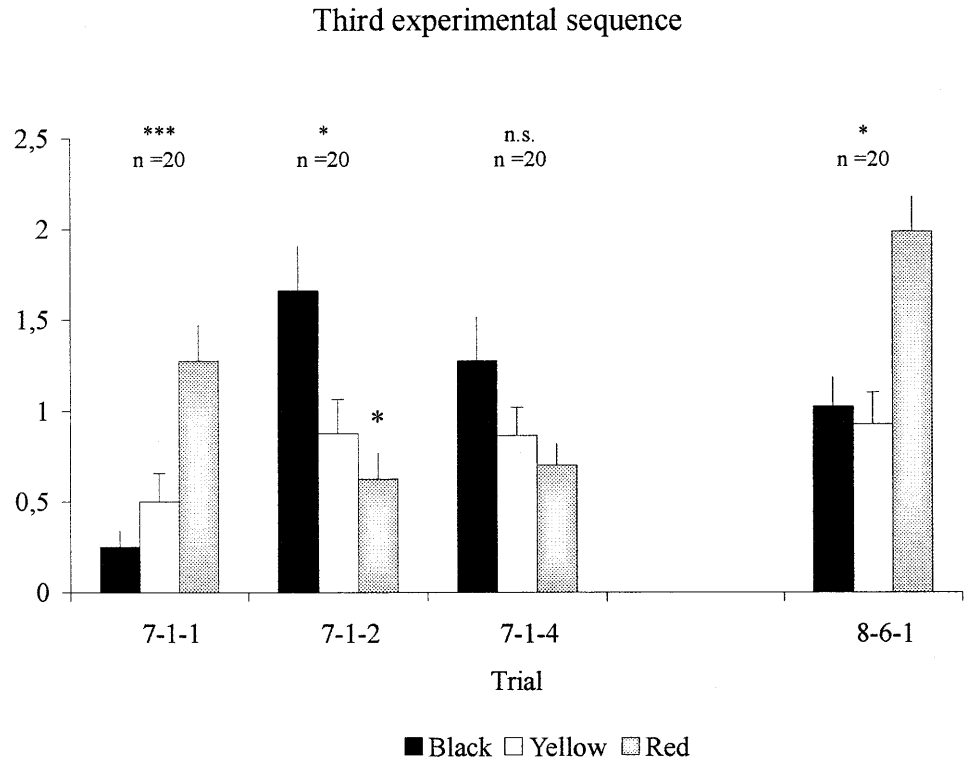

Fig. 3 The number of black, yellow, and red models consumed by reef fishes in the third experimental sequence. Trial code (x-y-z) represents: x: series number; y: experimental day, z: assay number during each series. *: color model coated with *Cacospongia* extract. In series 7, we offered fishes the three color models (assay 1), *Cacospongia* extract coated onto red models (assay 2), and the models without deterrents (assay 4). In series 8, we offered fishes the three color models without deterrents. Data are means $\pm$ SE, numbers above the bars denote sample size. Statistical significance was determined by using Hotelling's T2 test: *: $p < 0.05$; **: $p < 0.01$; ***: $p < 0.01$

Between the Piti Bombhole and Western Shoals fish populations, the conspicuousness of red and yellow colors may also differ.

At Piti Bombhole, we found learned avoidance of yellow models in the first experimental sequence, while the remaining trials showed no learned associations between color and feeding deterrence. At Western Shoals, we found learned avoidance of red models. Many factors that we did not control may have contributed to the more complex behavioral patterns we observed at Piti Bombhole, including hunger levels, turnover of the fishes present, and other dietary experiences. However, we have clearly demonstrated that populations of reef fishes are capable of learning to avoid colors associated with feeding deterrents. At Piti Bombhole, avoidance of the yellow models was retained for 5 d and may have affected subsequent trials. At Western Shoals, avoidance of the red models was not retained after 12 d. The high initial preference for red models may have contributed to less retention of this avoidance behavior.

In agreement with Tullrot (1991) and Marin *et al.* (1994), our study indicates that common colors of nudibranchs are recognizable by fishes and can be associated with noxious prey. The potential for learned avoidance of conspicuously colored nudibranchs provides additional evidence for aposematic coloration in nudibranchs (Edmunds 1991). Additional research is needed to determine if groups of nudibranchs that may be mullerian mimics show preferred colors in their designs and if these conspicuous color patterns are more effective at deterring fish predation than cryptic color patterns (Ros 1976; Rudman 1991; Sanchez-Jerez *et al.* 1994).

Trial code	*N*	Centered mean			T^2	*df*	*F*	*p*
		black	yellow	red				
7,1,1	20	−0.425	−0.175	0.600	1.830	3,17	10.371	0.000
7,1,2	20	0.608	−0.179	−0.429	0.783	3,17	4.437	0.018
7,1,4	20	0.329	−0.084	−0.246	0.348	3,17	1.970	0.157
8,6,1	20	−0.288	−0.388	0.675	0.900	3,17	5.098	0.011

Table 6 Result of Hotelling's T2 test on data from feeding preference of reef fishes in the third experimental sequence. Trial code (x, y, z) represents: x: series number; y: experimental day, z: assay number during each series. Centered mean ($X - k$): is the vector of differences between the sample means and the hypothesized constant k (the overall mean)

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